A preliminary study of defence behaviour of *Sipyloidea sipylus* (Westwood) using amphibian predators.

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Abstract

In a small number of trials, large nymphs of *Sipyloidea sipylus* (Westwood) were offered to three species of amphibians. Non-moving prey were ignored by all the predators. Phasmids which moved were eaten by only one species of amphibian. Results suggest that the chemical defences of *S. sipylus* offer little protection against amphibian predators but remaining immobile is an effective defence.

Key words

Phasmida, *Sipyloidea sipylus*, Nymphs, Behaviour, Defence, Amphibian.

Introduction

The stick insect *Sipyloidea sipylus* (Westwood, 1859) was originally described in the genus *Necroscia*. It is distributed in South East Asia (Westwood, 1859: 139; Redtenbacher, 1908: 544), including Taiwan (Shiraki 1935: 80), where it is bisexual. More recently, a parthenogenetic stock has been introduced and established in Madagascar (Chopard, 1954). The culture stock derived from Madagascar consists of parthenogenetic females with males occurring rarely (Urvoy, 1969). *S. sipylus* is a slender stick insect. The body colour of the adult is light brown with well developed pink wings and the body of the nymphs is green. In the immature stage, six well distinguished nymphal instars occur (Carlberg, 1981a & 1987), unlike the results observed by Browaeys-Poly (1973) in which seven nymphal instars were documented.

According to Robinson (1969), the anti-predator adaptations of insects could be classified into primary and secondary defence systems. In the case of *S. sipylus* the two main types of defence mechanisms for protection can be identified as follows: the primary defence system consists of grass mimicry (camouflage) and it also uses a chemical secretion as a secondary defence system (Carlberg, 1981b). The chemical secretion is produced by a pair of metathoracic glands which have been described by Rabozzi and Dazzini (1972). Other types of defence strategies have been observed in this insect such as thanatosis, running and the display of the pink wings by the adults (Carlberg, 1981b & 1981c).

In recent years, the defensive behaviour of some stick insects has been investigated using live predators as opposed to using forceps or a finger as stimuli which was the case in the studies mentioned above. Carlberg (1985a) studied the defence system of *Anisomorpha buprestoides* (Stoll) using a small rodent, *Rattus norvegicus* (Berkenhout) as a predator. The results obtained showed that the defensive secretion of *A. buprestoides* acts as a repellent against the rodents and therefore enables the stick insects to escape predation in most cases. In the same line of investigation, other stick insects such as *Extatosoma tiaratum* (MacLeay), *Carausius morosus* (de Sinéty), *S. sipylus* and *Eurycantha calcarata* Lucas were exposed to rodent predators to study their different defence behaviour (Carlberg, 1985b, 1985c, 1986, & 1989 respectively). In the study of the defence behaviour of fifth instar female nymphs of the stick insect *S. sipylus* using the rat *Rattus norvegicus* (Carlberg, 1986), four series of tests were performed with each rat. The results showed that only 20% of the insects were eaten when put in presence of a rat proving that the chemical defence of *S. sipylus* is more powerful than those of *E. tiaratum* and *A. buprestoides*. In the few instances where the nymphs of *S. sipylus* were killed, the rats had to make a large number of attacks before they were able to kill the stick insects. *S. sipylus* is thought to be a more or less ground living stick insect (Carlberg, 1981b & 1986) (although evidence of a more arboreal preference can be observed in culture stocks) implying that it would be exposed to many types of predators

(other insects, small mammals, lizards, frogs and birds) in its natural habitat.

Since the defence behaviour of *S. sipylus* seems to be very effective against rodent predators, as shown by Carlberg (1986), it would be interesting to test the effectiveness of the chemical secretions and other defence systems used by this insect when confronted with amphibian predators.

Material and methods

Animals

Female nymphs of *S. sipylus* were supplied by the Insectarium of Montreal, and kept in mesh cages at room temperature (about 20°C). All of the insects were fed with Red oak (*Quercus rubra* Linnaeus) and guava (*Psidium guajava* Linnaeus) leaves. Only the nymphs of the last (6th) instar were used in this experiment (body length: 78 ± 4mm).

Two males (initial mass of body: 139.9 & 163.2 ± 0.05 grams) and one female (initial mass of body: 193.4 ± 0.05 grams) adult Bullfrogs (*Rana catesbeiana* Shaw) were kept at the Ecomuseum, in an indoor reproduction of their natural North American habitat. The Bullfrogs were fed with domestic crickets *Acheta domesticus* (Linnaeus) (25 each every eight days) and also baby mice (4-5 grams each, given once every five days).

One male (initial mass of body: 38.0 ± 0.05 grams) American Toad (*Bufo americanus* Halbrook) and one male (initial mass of body: 45.0 ± 0.05 grams) Green Frog (*Rana clamitans* Latreille) were kept in separate 5 US Gallon glass tanks (40.5 x 25.4 x 20.3cm) at the Ecomuseum. Both were fed with domestic crickets (10 every eight days).

Experiments

All of the predators were fed a feeding cycle which was used previously by Carlberg (1985 b, c, 1986, 1989). The predators received food for eight days (prefeeding), then they were given no extra food on the ninth and tenth days (starvation). All of the tests were made during the eleventh day of the cycle. Three series of tests were performed with each of the predators in 5 US Gallon glass tanks. The insects used were used only once. The duration of the tests was 15 minutes.

The first predator-prey behavioural response (FPPBR) was recorded and this is defined as the first behaviour of the insect when introduced inside the glass tank containing the potential predator. The sum of all of the predator-prey behavioural responses during the 15 minutes of each test is defined as the total predator-prey behavioural response (TPPBR). The results are presented for each species. For each test, one nymph was introduced by hand into the cage where only one predator had been placed.

To clarify the data obtained in the three tests with *S. sipylus*, the same predators were used in four other tests as follows: 1 - with three live domestic crickets; 2 - with three freshly killed domestic crickets (killed by putting them in a freezer for ten minutes); 3 - with three treated crickets (done by shaking vigorously the live crickets and specimens of *S. sipylus* in a plastic bag for about 15 seconds); 4 - with one specimen of an unidentified stick insect of the genus *Bacteria* (PSG culture 152, from Venezuela), with average lengths of 85.2mm. The last four tests were done using exactly the same procedure as for the first three tests.

Results

The first reaction of the insects (FPPBR) when introduced inside of the cage with the predators is shown in Table 1. The nymphs walked forward in most of the cases (87%, N_{total} = 15) independently of the size or the species of the predator. Out of the total number of nymphs that moved in the FPPBR, three of them stimulated the predator by their movement

and as a result they were attacked and later eaten. The three times that the insects were ingested, they were attacked by the same predator in the three different tests, the Green frog. The manipulation of the nymphs triggered them to use their chemical defence in some, perhaps every, instance as a strong smell could be detected on the hands of the manipulator.

Bullfrogs	American toad	Green frog
89% ME*	66% ME*	100% ME
11% M*E*	33% M*E*	

Table 1. FPPBR for all the tests combined and for each of the species.
Note: M= moving prey; M*= non-moving prey; E= eaten by predator; E*= rejected by predator.

Bullfrogs	American Toad	Green Frog
100% M*E*	100% M*E*	100% ME

Table 2. TPPBR for all tests combined for each of the species.
Note: M= moving prey; M*= non-moving prey; E= eaten by predator; E*= rejected by predator.

Data was also recorded concerning the total predator-prey behavioural response (TPPBR). The results show that most of the insects (80%, N_{total} = 15) stayed immobile for more than half of the 15 minutes of the tests (Table 2). The other 20% indicates that the insect was moving in the cage with the predator for more than half of the test period. The three insects that were ingested by the Green frog are included in the 20%.

The Green frog is the only predator which has attacked and killed the nymphs of *S. sipylus* and it did so in the three tests. In the first test, the Green frog needed only one attack before killing the prey (50 seconds after the insect was introduced inside of the cage). In the second and third tests, the Green frog needed more attacks (three in each case) before killing the prey. The time of the attacks were identical for the second and third tests. The first attack came after only three seconds after the introduction followed by the second and third attack at four and five seconds from the introduction of the nymph inside of the cage. The attacks were timed using a slow motion video recording.

The position of the insects in the glass cages with the predators were recorded and listed in Table 3 and Table 4. As observed in the TPPBR, about 80% of the insects stayed in a state of immobility for more than half of the period of the tests. The glass walls of the tanks used for the tests seemed to have been preferred for the immobile position of the insects over the tank floor. The insects exhibited the immobile position in both the higher and the lower portions of the glass walls at about the same frequency. The body of the insects in the immobile position was always at an angle smaller than 90° from the vertical (the head of the insect being always higher than the abdomen).

Position	Occurrence	Frequency
Cage floor	2	0.12
Upper half of glass wall	7	0.41
Lower half of glass wall	6	0.35
Halfway between wall and floor	2	0.12

Table 3. Position of the insect inside of the glass tank during immobility (all tests combined).

Angle from vertical	Occurrence	Frequency
0-5°	9	0.69
6-45°	2	0.15
46-90°	2	0.15
>90°	0	0.00

Table 4. Angle of the insect's body when in the immobile position.

Another type of observation was recorded when the predators came within 5cm of the insect. This occurred five times (excluding the three times that the nymphs were eaten) and the results show that the insects, which were in a state of immobility every time, did not move at all from their position even if the predator moved closer for four of the five times. In the other instance, the insect moved away from the predator as soon as it got within 5cm. Three times the predator touched the insect accidentally and that created a movement of the insect away from the predator. The predators did not seem to be stimulated by the movement of the insects when they touched them.

One might ask if the size of the predator influenced the movement of the insects inside of the cage during the tests. Of all the tests with the Bullfrogs, two types of behaviour were observed: an immobile position was maintained by the insect for 95.8% of the total duration of the tests with the Bullfrogs, the other 4.2% represented a moving behaviour by the insect (which was mostly when the insect was first introduced in the cage). The results observed with the insects confronted with the American toad were almost identical as 96.0% of the total time spent by the insect was in a state of immobility. The Bullfrogs and the American toad used in this experiment were the two species of predators with the wider difference in body mass suggesting that the behaviour of the insects was not influenced by the size of the predators at this level. No case of autotomy was recorded in any of the tests.

To clarify the reasons why four out of the five predators used did not attack the nymphs of *S. sipylus*, four subsequent tests were done using different prey. In table 5 we can see that all of the predators ate at least one cricket including the treated crickets. The treatment with the chemical secretions of *S. sipylus* did not seem to keep the predators from eating the crickets as three out of five predators attacked them. It is important to notice that the dead crickets were never attacked. Only the Green frog attacked the specimen of stick insect presented to it which is consistent with the previous results.

Predators	Live crickets	Dead crickets	Treated crickets	*Bacteria* sp.
Green frog	X	-	X	X
American toad	X	-	-	-
Bullfrog 1 (female)	-	-	X	-
Bullfrog 2 (male)	X	-	-	-
Bullfrog 3 (male)	X	-	X	-

Table 5. Prey attacked by the predators (X) in the subsequent tests.

Discussion

The results of the FPPBR show a bias towards the nymphs of *S. sipylus* being rejected both when moving and in a state of immobility, which is similar to the results found by Carlberg (1986). In the majority of the cases, the nymphs of *S. sipylus* walked for a few seconds after being introduced inside the cage with the predator. This behaviour could probably be explained by the fact that the insects were somewhat stressed by the movement from one environment (rearing cage) to another (test cage) by hand. Even if 87% of the insects moved for a few seconds after their insertion, the predators did not seem to be stimulated visually by this movement (except for the Green frog).

Keeping in mind that the amphibian predators used in this study were fed no food for the two days preceding the tests, one might wonder why only one out of the five predators ate the nymphs. With the use of sensitive visual detector cells, amphibians are able to evade oncoming predators and catch fast moving prey (Mitchell, Mutchmor & Dolphin, 1988). Theoretically, the initial movement by the nymphs should have stimulated the predators to attack. However, the results show otherwise which suggests that something stopped the amphibian predators from attacking the insects presented to them. It was first hypothesized that the chemical defence was effective against amphibian predators but the results obtained from the test with the treated crickets show otherwise. In fact, the three predators which attacked the treated crickets also ate them. If the chemical defence was noxious or repelling, the predators would have rejected the prey but the presence of the secretion of *S. sipylus* on the bodies of the crickets did not seem to stop the predators from eating them, therefore, the initial hypothesis that the chemical defence of *S. sipylus* has a strong repelling effect on the amphibian predators is ruled out. All of the predators ate at least one cricket proving that they were hungry.

Because none of the dead crickets were attacked, we can strongly suggest that immobile behaviour exhibited by prey such as *S. sipylus* would be a great adaptive value against potential attacks from amphibian predators. The fact that all the predators attacked crickets in at least one of the tests show that these predators associate the shape and the behaviour of the crickets with food. On the other hand, the predators did not associate the stick insects with food. In the wild, these North American species of amphibians do not come in contact with stick insects often because only a few rare species exist in their range. From the results of this study, we feel that it is more a combination of behaviour (immobility or vegetation mimicry) and unusual shape to the predators that kept the nymphs of *S. sipylus* from being attacked and eaten.

The reason why the Green frog ate the nymphs is still unknown. We could suspect that the Green frog is a more aggressive species than the others but it could also be attributed to a more aggressive individual of that species. Another way of explaining the reason why the green frog ate all three nymphs in the three tests is by assuming trial and error. The first attack by the green frog in the first test came 50 seconds after introduction. The nymph had moved a little for the first few seconds of the introduction and then stayed in a state of immobility on one of the glass walls until it started to move again towards the motionless green frog (after about 40 seconds). The attack came only after the nymph touched the green frog with its antenna and then with one of its front legs. If the insect had stayed in a state of immobility for the rest of the experiment, it can be hypothesized by the behaviour of the other predators that the Green frog would not have attacked the nymph. For the green frog, the drive to feed on the nymph probably overcame the unusual shape of the insect especially after the insect touched the frog. The result was that the green frog attacked and ate the insect. After that first successful trial, the green frog learned that the nymph was good to eat and this would explain the very fast attacks on the nymphs for the subsequent two tests. More testing with a larger sample is necessary in order to answer all these questions.
When comparing the TPPBR, a definite bias towards the state of immobility (M^*; 80% of times, N_{total} =15) and the rejection of the non moving prey (M^*E^*; 100%) was observed. These results are also very similar to the ones obtained by Carlberg (1986) which shows that the defence strategy of the nymphs is usually the same, independent of the kind of predator (rodent or amphibian) it is facing.

The position of the immobile body of the nymphs was recorded; this revealed a preference for a vertical position (69% within 5° from the vertical, N=13) on one of the glass walls (76%, N=17). This defence behaviour is certainly advantageous for this insect which can make it look like a part of the vegetation.

The nymphs of *S. sipylus* did not move (80% of times, N=5) away from the predators even if the predators moved within 5cm of them. This suggests that it is advantageous for the insects to stay immobile and thus not to create a visual stimulation. It could also be disadvantageous because being so well disguised, the insects could potentially be stepped on by the predators causing injury or death. No case of autotomy occurred in this study which agrees with the low autotomy frequencies found by Carlberg (1981b, 1986).

According to Carlberg (1986) *S. sipylus* is a more or less ground living insect which is exposed to a large number of different predators, e.g. other insects, small mammals, lizards, frogs and birds in its natural habitat. With a combination of defence mechanisms such as grass mimicry and chemical secretion, we can say that this species of stick insect is very well adapted against both rodents (Carlberg, 1986) and amphibian predators.

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